Attachment to Preliminary Amendment dated June 12, 2002

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Page 4, Paragraph Beginning at Line 10

Fig. 2 is an alignment of the deduced amino acid sequence of *CYP706B1* (SEQ ID NO.:2) of *G. arboreum* with those of *CYP706A4* (SEQ ID NO.:3) and *CYP706A5* (SEQ ID NO.:4) of *A. thaliana*. Consensus sequences discussed are underlined. The region used to synthesize the degenerate primer is also underlined. Its sequence in *CYP706B1* turned out to be slightly different from the primer.

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Cloning of cDNA - A degenerate primer (SEQ ID NO.:5)

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Probes were generated from total RNAs isolated from developing seeds of the glanded and glandless *G. hirsutum* cultivars, respectively, and the first strand cDNA was synthesized as previously described (Meng et al (1999) *J. Nat. Prod.* **62**, 248-252). The cDNAs were ^{32}P-labeled using a random DNA labeling kit (Takara, Dalian, China), and used for dot-hybridizations. Clones *LP132* and *LP64* showing preferential hybridization with probes of glanded seeds were selected and sequenced by the dideoxynucleotide chain termination method. Specific primers LP132F (SEQ ID NO.:7) [5¢-TGACTGATCATGAGAAGCT (sense)] and LP132R (SEQ ID NO.:8) [5¢-

GTGCTGGAGATTTGATGGT (reverse)] based on the sequence of *LP132* were then used for screening the *G. arboreum* cDNA library by using a PCR 96-well plate method (Liu et al (1999) *Mol. Plant Microbe Interact.* **12**, 1095-1104). A cDNA clone, *CYP706B1*, was then isolated and sequenced (See SEQ. ID. NO. 1; GenBank/EBI Data Bank Accession No. AF332974).

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Pericarp (approx. 3 mm thick) was peeled from bolls with a blade. Total RNAs were isolated from tissues or from suspension cultured cells by a cold phenol method, and the transcripts were analyzed by RT-PCR with primers LP132F and LP132R for *CYP706B1* (position 1433 ~ 1689), 97400 (SEQ ID NO.:9) [5¢-CACATCC(AC)TTCGATTCCGAC (sense)] and 97T580 (SEQ ID NO.:10) [5¢-AGGCTTAAATGGTGGGTGGT (reverse)] for *CAD1-C* (position 398 ~ 610), and H3F (SEQ ID NO.:11) [5¢-GAAGCCTCATCGATACCGTC (sense)] and H3R (SEQ ID NO.:12) [5¢-CTACCACTACCATCATGTC (reverse)] for the histone gene *his3* (positions 95 ~ 526). For Northern analysis, 10 mg of RNA per lane were separated by electrophoresis, blotted onto a nitrocellulose membrane, and the blots were hybridized with ^{32}P labeled DNA probes of either *CYP706B1* (see above) or *CAD1-C1* (Liu et al (1999) *Mol. Plant Microbe Interact.* **12**, 1095-1104). After hybridization and washing, the blots were exposed to X-ray film for 2 days.

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Expression in yeast cells and enzyme assay - The yeast *Saccharomyces cerevisiae* strain W(R), which overexpresses the yeast cytochrome P450 reductase when grow on galactose, and the expression vector pYeDP60 were provided by D. Pompon (Pompon et al (1996) *Methods Enzymol.* **272**, 51-64). The cDNA of CYP706B1 was modified by PCR with a 5'-terminal primer (SEQ ID NO.:13) 5¢-GGGTACCATGTTGCAAATAGCTTTCAG (sense), in which a *Kpn* I site was introduced, and a 3'-terminal primer (SEQ ID NO.:14) 5¢-GGGAGCTCTTACTTCATATAGTGCTGGA (reverse), in which a *Sac* I site was introduced. PCR was conducted on plasmid DNA by using Pyrobest™ DNA polymerase (TaKaRa). After digestion with the restriction enzymes, the fragment was inserted into pYeDP60. Plasmid DNA was introduced into yeast cells by a LiAc method, transformed yeast cells were then selected, cultured, and induced, and microsomes were prepared following a high density procedure (Pompon et al (1996) *Methods Enzymol.* **272**, 51-64).

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Sequence analysis revealed several structural motifs characteristic of eukaryotic P450s (Fig. 2). The highly conserved heme-binding motif FxxGxRxCxG (Chapple, C. (1998) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 49, 311-343) was found in CYP706B1 (SEQ ID NO.:15) as FGSGRRMCAG, 73 amino acid residues from the C-terminus. In most plant P450s, there is a proline residue immediately after the invariant heme-binding cysteine (Schalk et al (1999) *Biochemistry* 38, 6093-61103); however, in CYP706B1, this

proline is replaced by alanine. The proline-rich region immediately after the N-terminal signal anchor sequence (Nelson, D. R., and Strobel, H. W. (1988) *J. Biol. Chem.* 263, 6038-6050), with a consensus of (SEQ ID NO.:16) (P/I)PGPx(G/P)xP (Schalk et al (1999) *Biochemistry* 38, 6093-61103), was completely conserved in this cotton P450 as PPGPRGLP (SEQ ID NO.: 17). In addition, the threonine-containing pocket for binding an oxygen molecule, with a consensus of (SEQ ID NO.:18) (A/G)Gx(D/E)T(T/S) (Durst, F., and Nelson, D. R. (1995) *Drug Metab. Drug Interact.* 12, 189-206), was also found (as GGTDTT) (SEQ ID NO.:19).